Vol. 501: 99–111, 2014
doi: 10.3354/meps10690

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published March 31

FREE ACCESS

Diel pCO_2 oscillations modulate the response of the coral *Acropora hyacinthus* to ocean acidification

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ABSTRACT: To investigate the effect of diel variation in pCO_2 on coral calcification, branches of *Acropora hyacinthus* were collected in 2 habitats (upstream and downstream in a unidirectional flow) in a shallow back reef in Moorea, French Polynesia, where different diel amplitudes of pCO_2 oscillation were expected. Corals were maintained for 6 wk under different pCO_2 regimes (constant versus oscillatory), each delivered in 3 configurations: constant conditions of 400 µatm, 700 µatm, and 1000 µatm pCO_2, or oscillatory conditions varying daily from 280 to 550 µatm, 550 to 1000 µatm, or 400 to 2000 µatm, with minima and maxima during the day and night, respectively. Calcification rates in all treatments tended to increase over time, and the interaction between Time and pCO_2 regime (i.e. constant versus oscillating) was significant (or close to significant) for upstream corals due to higher calcification in oscillatory pCO_2. A significant pCO_2 regime effect was detected in the highest pCO_2 for downstream corals, with higher calcification in the 400 to 2000 µatm oscillatory pCO_2 treatment compared to the 1000 µatm constant pCO_2 treatment. After 6 wk, calcification of *A. hyacinthus* was affected significantly by habitat, the pCO_2 level, and the pCO_2 regime. Calcification generally was reduced by high pCO_2 and was ≥21% greater in 400 to 2000 µatm oscillatory pCO_2 versus 1000 µatm constant pCO_2 treatment. Increased calcification in the 400 to 2000 µatm oscillatory pCO_2 treatment suggests that natural diel oscillations in pCO_2 could play a role by reducing the locally negative effects of rising pCO_2 associated with ocean acidification on coral calcification.

KEY WORDS: Calcification · Corals · Acclimation

INTRODUCTION

Ocean acidification (OA) is one of the main threats to the future of scleractinian corals (Hoegh-Guldberg et al. 2007), which are the ecosystem engineers of the world's largest biogenic calcium carbonate framework. OA is caused by the dissolution of atmospheric CO_2 into the ocean, which alters the ocean carbonate chemistry system, depressing the concentration of carbonate ions, increasing the concentration of bicarbonate ions, and decreasing pH (Feely et al. 2004). Empirical evidence (e.g. Langdon et al. 2000) is providing a comprehensive understanding of the impacts of OA on coral reefs (Kleypas & Yates 2009), with studies reporting a decrease in coral calcification ranging from 0 to 100% for a doubling of present-day pCO_2 (Erez et al. 2011), with a mean decrease in calcification of ~10 to 20% (Chan & Connolly 2013, Comeau et al. 2013a).

However, most organism-based experimental studies of the effects of OA on corals have employed pCO_2 conditions (generally constant) that mimic the present conditions and those expected by the end of the century (e.g. Reynaud et al. 2003). Diurnal oscillations of pCO_2 have been taken into account only in few studies that looked at the effects of OA on coral communities incubated under conditions mimicking present pCO_2 (or pH) daily oscillations and present pCO_2 oscillations on which an additional pCO_2 increase was applied (Jokiel et al. 2008, Andersson et

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al. 2009). Recently, progress also has been made in the development of *in situ* incubation chambers that are designed to reproduce natural oscillations in pCO_2 under ambient and acidified conditions (Kline et al. 2012). The effects of oscillations in pCO_2 (i.e. constant versus oscillating treatments) on corals have been tested in only 1 study (Dufault et al. 2012) in which coral recruits were exposed to constant and oscillatory pCO_2 simulating natural daily variations in pH and pCO_2 on shallow coral reefs. Dufault et al. (2012) found that corals respond in different ways to each condition with the highest rates of calcification occurring under oscillating pCO_2 conditions.

Natural daily variations in seawater pH on some coral reefs already create conditions that are more acidic for short periods than those created in most perturbation experiments designed to simulate pCO_2 conditions expected to occur in the oceans of the future (e.g. Santana-Casiano et al. 2007, Bates et al. 2010, Hofmann et al. 2011). In Moorea, French Polynesia, Hofmann et al. (2011) described the pH of seawater on a shallow fringing reef for 30 d, and reported a mean of 8.072, a maximum of 8.118 at ~19:30 h, and a minimum of 8.017 at ~10:00 h. Using seawater conditions typical for Moorea in 2012 (27°C, salinity = 35, and a total alkalinity = 2380 µmol kg^{-1}; Comeau et al. 2013a), the aforementioned changes in seawater pH correspond to daily oscillations in pCO_2 of ~110 µatm. Higher diel variation in seawater pH has been detected for other tropical reefs, including the reef terrace at Palmyra Atoll where pH varies 0.25 over 24 h (Hofmann et al. 2011), corresponding to a diel change in pCO_2 of ~350 µatm. On a spatial scale of ~700 m across a single reef, different magnitudes of pH oscillations have also been reported, with offshore sites exhibiting less variability than back reef sites where the residence time of seawater is greater (Ohde & van Woesik 1999).

In the future, OA has been predicted to result in diel variations in seawater pCO_2 that are larger than those occurring currently. For example, Shaw et al. (2013) proposed that increases in pCO_2 on reef flats on the Great Barrier Reef (Australia) will not be a linear function of atmospheric pCO_2, but rather will be manifest as a 3-fold amplification of the routine diel variation in pCO_2, thereby creating nocturnal pCO_2 as high as 2100 µatm by the end of this century (Shaw et al. 2013). Such extremes are hypothesized to arise from an increase in the Revelle factor (Revelle & Suess 1957, Sabine et al. 2004), which describes how pCO_2 in seawater varies for a given change in dissolved inorganic carbon (DIC). An increase of the Revelle factor describes the disequilibrium in the ratio between DIC and total alkalinity (A_T) caused by both the long-term increase in oceanic CO_2 and by calcification, dissolution, respiration, and photosynthesis over time scales pertinent to reef metabolism (i.e. hours to days). Since the capacity for oceans to absorb CO_2 from the atmosphere is inversely proportional to the value of the Revelle factor, higher values of the Revelle factor represent limited CO_2 uptake from the atmosphere.

To study the potential for diel pCO_2 oscillations to affect the response of corals to OA and to explore the effects of reef habitat origin on the response of corals to oscillatory pCO_2, we quantified the response of corals to oscillatory pCO_2. Colonies from an 'upstream' and 'downstream' habitat along a flow gradient across a back reef habitat in Moorea, French Polynesia (e.g. Rosman & Hench 2011) were compared to evaluate the potential role of habitat on the response to exposure to oscillatory pCO_2 of varying magnitudes. Our study was performed using the coral *Acropora hyacinthus*, which is found commonly in both upstream and downstream habitats on the back reef of Moorea. We tested the effects of oscillatory pCO_2 regimes corresponding to those occurring presently (280–550 µatm), to one scenario expected by the end of the current century (550–1000 µatm) and to one scenario of extreme diel oscillation in seawater pCO_2 (400–2000 µatm). Oscillating pCO_2 treatments were contrasted with constant pCO_2 treatments representing the average daily pCO_2 encountered in the 3 oscillating treatments. The impact of constant versus oscillating pCO_2 on calcification was investigated on coral fragments that were maintained during a 6 wk incubation under differing pCO_2 treatments.

MATERIALS AND METHODS

Habitats and coral collection

Acropora hyacinthus branches were collected from 2 habitats on the north shore of Moorea (Fig. 1), at the end of August 2012. Corals were sampled in 2 habitats along a gradient of flow over the back reef with the rationale that offshore seawater comes over the reef crest, and seawater DIC is modified by benthic community metabolism (Ohde & van Woesik 1999). An 'upstream' habitat was sampled in the back reef, ~20 m shoreward of the reef crest, where carbonate chemistry conditions were expected to be stable throughout the day (after Ohde & van Woesik 1999). A 'downstream' habitat was sampled in the lagoon

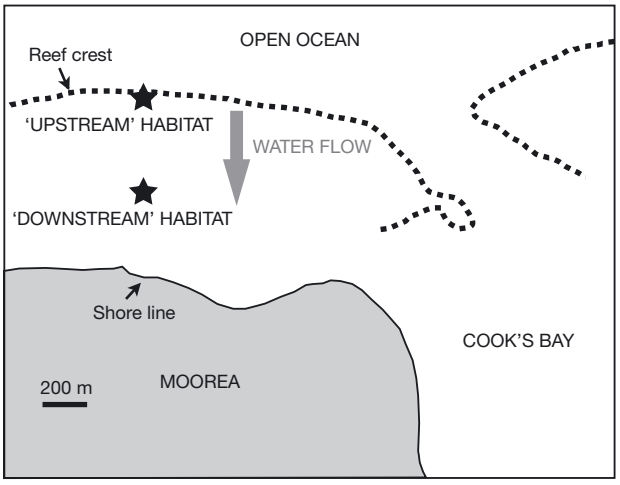

Fig. 1. Location of the 2 habitats from which *Acropora hyacinthus* nubbins were collected. Habitat 1 ('Upstream') was located on the back reef, ~20 m from the reef crest, and habitat 2 ('Downstream') was located in the lagoon ~400 m shoreward from the reef crest

~400 m shoreward of the reef crest, where larger daily oscillations in pH were expected (Ohde & van Woesik 1999). To quantify the magnitude of the diel oscillation of seawater DIC conditions, 2 SeaFET pH sensors (Martz et al. 2010, SIO/UCSD) were deployed during the incubation at 2 m depth at the collection sites to record pH from 11 September to 20 October 2012. Calibrations of the pH sensors were conducted pre- and post-deployment by placing them in tanks in which pH was measured spectrophotometrically (SOP 6b, Dickson et al. 2007). Seawater samples were also taken monthly <1 m from the SeaFET pH sensors to determine pH and to quantify the extent to which the sensors drifted from calibrated values.

We collected 42 branches (~4 cm long) of *Acropora hyacinthus* from each habitat at ~1 to 2 m depth. The branches were transported to the Richard B. Gump South Pacific Research Station and glued to plastic bases (4 × 4 cm) with Z-Spar (A788 epoxy) to form nubbins. Nubbins were placed in a seawater table for 2 d to recover from preparation. This recovery period provided sufficient time for the epoxy to cure and for tissue regeneration to begin, but not enough time to acclimate to the laboratory conditions and mask potential effects of their habitat origins. Approximately 2 wk (or more) are required for corals to acclimate to changes in environmental conditions (e.g. light, flow, and temperature) associated with collection and preparation for experiments (Anthony & Hoegh-Guldberg 2003, Tentori & Allemand 2006, Form & Riebesell 2012).

Treatments and regulation of pCO_2

Incubations were performed in 12 mesocosms (each ~150 l) in which light was provided by 75W LED modules (Sol White LED Module, Aqua Illumination) that delivered ~500 µmol quanta m^{-2} s^{-1} on a 12:12 h light:dark cycle. Temperature was kept at 27°C, which is close to the seawater temperature in the lagoon of Moorea in August (Edmunds et al. 2010). Seawater was supplied from 12 m depth in Cook's Bay, filtered (pore size ~100 µm), and delivered to the tanks at ~150 ml min^{-1}. The delivery of fresh seawater prevented the total alkalinity in the tanks from being modified by coral metabolism.

The mesocosms were used to create a contrast between constant pCO_2 and diel oscillating pCO_2. Six treatments were created in duplicate (i.e. 2 tanks $treatment^{-1}$) and were used to independently incubate 7 corals from 1 habitat in 1 tank and 7 corals from the second habitat in the duplicated tank to allow an inferential contrast between habitats. The 3 constant pCO_2 treatments consisted of (1) present day pCO_2 (400-constant: 400 µatm), (2) a pCO_2 value commonly predicted by the end of the current century under a representative concentration pathway (RCP) of 6.0 (Moss et al. 2010; 700-constant: ~700 µatm), and (3) a pessimistic pCO_2 projection expected by the end of the 21st century under RCP 8.5 (1000-constant: ~1000 µatm). The 3 oscillatory pCO_2 treatments mimicked diel variation occurring presently (e.g. Hofmann et al. 2011) versus diel variations of an enhanced magnitude (sensu Shaw et al. 2013), and these oscillations were created to evenly bracket the constant pCO_2 levels used in the stable treatments. The oscillations applied high pCO_2 at night and low pCO_2 during the day as occurs naturally on shallow reefs due to benthic respiration and photosynthesis. Oscillating pCO_2 treatments consisted of 3 treatments: (1) 280–550-oscillating, mimicking present conditions (280 µatm during the day to 550 µatm at night), (2) 550–1000-oscillating, representing the conditions expected by the end of the century on a reef with moderate diel oscillations (550 µatm during the day to 1000 µatm at night), and (3) 400–2000-oscillating, representing extreme conditions unlikely to occur in Moorea. The extreme scenario was based on the assumption that large diel oscillations in pCO_2 would occur in some coral reefs, as some coral reefs are already experiencing diel oscillations in pCO_2 as large as 150 to 1325 µatm (Shaw et al. 2012). This assumption was confirmed by a recently published study showing that the tested extreme diel oscillations (400 µatm during the day to

2000 µatm at night) could occur by the end of the current century on some reefs (Shaw et al. 2013). To create oscillatory pCO_2 conditions, coral nubbins were transferred daily at 07:00 h from high pCO_2 to low pCO_2 treatment, and at 19:00 h from low pCO_2 treatment to high pCO_2 treatment. Corals in all 3 constant treatments also were transferred from one duplicated tank to the other at 07:00 h and 19:00 h to serve as a procedural control for the manipulation.

CO_2 treatments were created by bubbling ambient air, CO_2-enriched air, or CO_2-depleted air into the tanks. CO_2-enriched air was created using a solenoid-controlled gas regulation system (Model A352, Qubit Systems) that mixed pure CO_2 and ambient air to achieve the desired pCO_2. CO_2-depleted air was obtained by scrubbing CO_2 from ambient air by passing it through soda lime, and was delivered to incubation tanks by 10 cm long airstones. The flow of air and CO_2-manipulated (CO_2-enriched and CO_2-depleted) air into each tank was adjusted independently using needle valves to correct for deviations in seawater pH from the targeted values. Departures in pH from targeted values were detected by measurements of seawater conditions in the tanks at 07:00 h and 19:00 h (described in the next section).

Carbonate chemistry

Seawater pH was measured at 07:00 h and 19:00 h in each tank, using a pH meter (Orion, 3-stars mobile coupled with a Mettler DG 115-SC pH electrode) calibrated every 2 d on the total scale using Tris/HCl buffers (Dickson). Calibrations were conducted every 2 d because the pH probe was stable and drifted only 0.1 mV between calibrations, corresponding to a pH variation of <0.003. Total alkalinity (A_T) of the seawater in the tanks was measured every 2 d using open-cell, potentiometric titrations and an automatic titrator (T50, Mettler-Toledo). A_T was calculated using a modified Gran function, as described by Dickson et al. (2007), and titrations of certified reference materials provided by A.G. Dickson (batch 105) yielded A_T values within 2.8 µmol kg^{-1} of the certified value (SE = 3.6 µmol kg^{-1}; n = 19). Salinity in the tanks was measured weekly using a portable conductivity meter (YSI 63) and remained constant at 36.0 during the 6 wk incubation. A_T, pH_T, temperature, and salinity were used to calculate the carbonate chemistry parameters using the Seacarb package (Lavigne & Gattuso 2012) running in R software (R Foundation for Statistical Computing). The Lueker et al. (2000) constants for K_1 and K_2, the Perez & Fraga (1987) constant for K_f, and the Dickson (1990) constant for K_s were used for all of the calculations.

Calcification

Seven nubbins from upstream and downstream habitats were placed randomly in each of the 6 treatments, and calcification was measured over the 6 wk period using buoyant weighing (Spencer-Davies 1989). Buoyant weight (±1 mg) was recorded at the beginning of the incubation and weekly thereafter to test for acclimation to the treatments. The difference in buoyant weight between each week of incubation was converted to dry weight using the density of aragonite (2.93 g cm^{-3}) and used to calculate weekly rates of calcification. Calcification was normalized to surface area of the coral tissue (mg cm^{-2} d^{-1}) determined using wax dipping (Stimson & Kinzie 1991).

Statistical analysis

The assumptions of normality and equality of variance were evaluated through graphical analyses of residuals using R software. Due to logistical constraints, corals from 1 treatment and 1 habitat were incubated in 1 tank, which is a pseudoreplicated design (Hurlbert 1984). Nevertheless, care was taken to maintain tight control of the carbonate chemistry, temperature, and light in the tanks (see Table 1), which reduced the potential for these factors to confound the contrast of interest (i.e. the problem defined as pseudoreplication). In addition, incubations were performed in large (150 l) open-flow tanks, which reduced the potential for individual corals to affect others or to alter the seawater chemistry through their metabolism. For these reasons, we propose that the potential for pseudoreplication effects (Hurlbert 1984) to affect the interpretation of the outcome of our experimental design was unlikely to be important in our experimental design, and therefore the nubbins were treated as individual replicates.

To test for acclimation to the treatments, differences between pCO_2 regimes (constant versus oscillating pCO_2) and changes over time (i.e. weeks of incubation) for area-normalized calcification were investigated for each pCO_2 using repeated-measures ANOVA with pCO_2 regime as the between-subject effect and time as a within-subject effect. Based on the rationale that corals were fully acclimated to the

treatment after 5 wk (e.g. Tentori & Allemand 2006), a 3-way mixed model ANOVA was employed to test the effect of origin habitat, average daily pCO_2 condition (i.e. 400, 700, and 1000 µatm), and pCO_2 regime (constant versus oscillating, nested effect) on the calcification during the last week of incubation.

RESULTS

Carbonate chemistry at sampling sites and in mesocosms

In situ measurements of seawater pH at the 2 habitats showed average mean daily pH of 8.079 ± 0.007 upstream (~371 pCO_2 µatm, for an A_T = 2380 µmol kg^{-1} and 27°C) and 8.075 ± 0.007 downstream (± SD, n = 40, ~376 pCO_2 µatm); these values were significantly different (paired *t*-test, p = 0.044) despite a very small difference in absolute value (0.004 pH units). Over 39 d, pH_T exhibited a mean daily maximum of 8.111 ± 0.013 (~338 pCO_2 µatm) and a mean minimum of 8.047 ± 0.017 (~407 pCO_2 µatm) in the upstream habitat, and a mean daily maximum of 8.119 ± 0.016 (~330 pCO_2 µatm) and a mean minimum of 8.041 ± 0.010 (~415 pCO_2 µatm) in the downstream habitat (Fig. 2; all ± SD, n = 39). Mean maxima were significantly different between habitats (paired *t*-test, p = 0.019), but mean minima were not (paired *t*-test, p = 0.087).

In the mesocosms, control of pCO_2 was precise, with mean daily pCO_2 in the oscillating treatments close to the constant treatment (Table 1). The mean daily pCO_2 was 366 µatm in the 400-constant versus 382 µatm in the 280–550-oscillating treatment, 638 µatm in the 700-constant versus 745 µatm in the 550–1000-oscillating treatment, and 983 µatm in the 1000-constant versus 1102 µatm in the 400–2000-oscillating treatment. Differences between constant and oscillating pCO_2 were not statistically different for 400-constant versus 280–550-oscillating treatments (Mann-Whitney test, p = 0.787) and 1000-constant versus 400–2000-oscillating treatments (Mann-Whitney test, p = 0.888). The difference between means was significantly different for 1000-constant versus 400–2000-oscillating treatments (Mann-Whitney test, p = 0.039), although it is unlikely to have any biological implications.

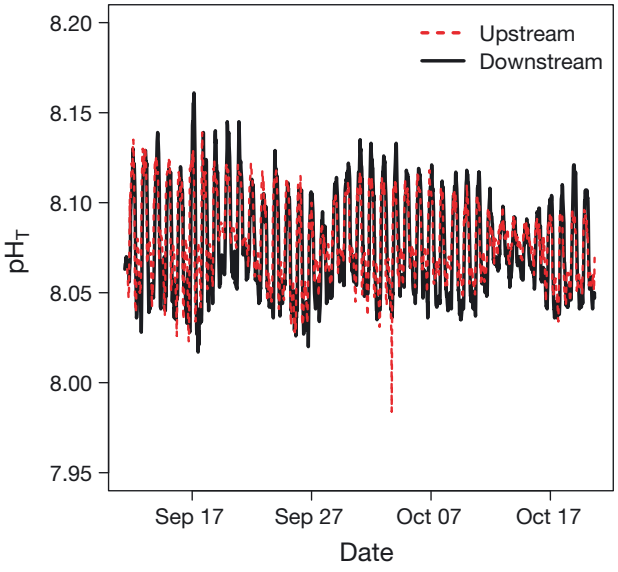

Fig. 2. Measurements made by SeaFET pH sensors deployed at the 2 habitats. pH measurements were made from 11 September to 20 October 2012 at a frequency of 48 measurements d^{-1} (5.5×10^{-4} Hz)

Table 1. Mean carbonate chemistry during the 6 wk incubation. Mean ± SE (n = 162) pCO_2 and Ω_{arag} were calculated from pH_T, total alkalinity (A_T), temperature, and salinity using the R package seacarb (Lavigne & Gattuso 2012). Salinity remained constant at 36.0 during the 6 wk incubation in all tanks. 'Mean oscillating pCO_2' shows the mean pCO_2 experienced by the corals in the oscillating CO_2 treatments (high–low pCO_2 12:12 h daily cycle). na: not applicable

Treatment	Targeted pCO_2 (µatm)	pCO_2 (µatm)	A_T (µmol kg^{-1})	pH_T	Ω_{arag}	Temp (°C)	Mean oscillating pCO_2 (µatm)
400-constant	400 constant	366 ± 4	2335 ± 2	8.07 ± 0.004	3.89 ± 0.03	27.0 ± 0.04	na
700-constant	700 constant	638 ± 11	2327 ± 1	7.88 ± 0.006	2.73 ± 0.03	26.9 ± 0.04	na
1000-constant	1000 constant	983 ± 15	2328 ± 2	7.71 ± 0.006	1.98 ± 0.02	27.0 ± 0.03	na
280–550-oscillating	280 – day	257 ± 5	2326 ± 2	8.20 ± 0.006	4.73 ± 0.05	27.0 ± 0.06	382 ± 7
	550 – night	507 ± 9	2325 ± 2	7.96 ± 0.006	3.19 ± 0.03	27.0 ± 0.03	
550–1000-oscillating	550 – day	507 ± 9	2325 ± 2	7.96 ± 0.006	3.19 ± 0.03	27.0 ± 0.03	745 ± 13
	1000 – night	983 ± 15	2328 ± 2	7.71 ± 0.006	1.98 ± 0.02	27.0 ± 0.03	
400–2000-oscillating	400 – day	366 ± 4	2335 ± 2	8.07 ± 0.004	3.89 ± 0.03	27.0 ± 0.04	1102 ± 14
	2000 – night	1839 ± 24	2347 ± 2	7.47 ± 0.007	1.22 ± 0.02	27.0 ± 0.04	

Acclimation to pCO_2 treatments

During the 6 wk incubation, no mortality of corals from upstream and downstream habitats occurred. Mean calcification over the whole period was positive in corals from habitats in all treatments (Fig. 3).

In upstream corals, calcification rates in all treatments were dependent linearly on time with slopes ≥0 mg $CaCO_3$ cm^{-2} d^{-1} wk^{-1} (Fig. 3a–c). In the contrast between 400-constant and 280–550-oscillating in upstream corals (Fig. 3a), pCO_2 regime did not have a significant effect ($p = 0.672$, Table 2) but there was a significant effect of Time ($p < 0.001$, Table 2). There was also a trend toward an effect of the Time × pCO_2 regime interaction ($p = 0.064$, Table 2), indicating that the response of calcification to the treatments tended to differ over time. Similarly, in the contrast between 700-constant and 550–1000-oscillating (Fig. 3b), pCO_2 regime did not have a significant effect ($p = 0.721$, Table 2), Time had a significant effect ($p < 0.001$, Table 2), and there was a trend toward a significant effect of the Time × pCO_2 regime interaction ($p = 0.070$, Table 2). The pCO_2 regime also did not have a significant effect on calcification ($p = 0.419$, Table 2) between 1000-constant and 400–2000-oscillating treatments (Fig. 3c). In contrast to the other configurations, both time and the interaction Time × pCO_2 were significant ($p < 0.001$ and $p = 0.041$, respectively; Table 2) due to the steeper increase in calcification over time for 400–2000-oscillating compared to the 1000-constant treatment.

In downstream corals, calcification was positive and linearly dependent on time in all treatments, except 280–550-oscillating and 700-constant, where the relationships were best fit by second-order polynomial regressions (Fig. 3d,e). In the comparison between 400-constant and 280–550-oscillating (Fig. 3d), calcification was not significantly affected by the pCO_2 regime ($p = 0.843$, Table 2) but was significantly affected by both Time and the Time ×

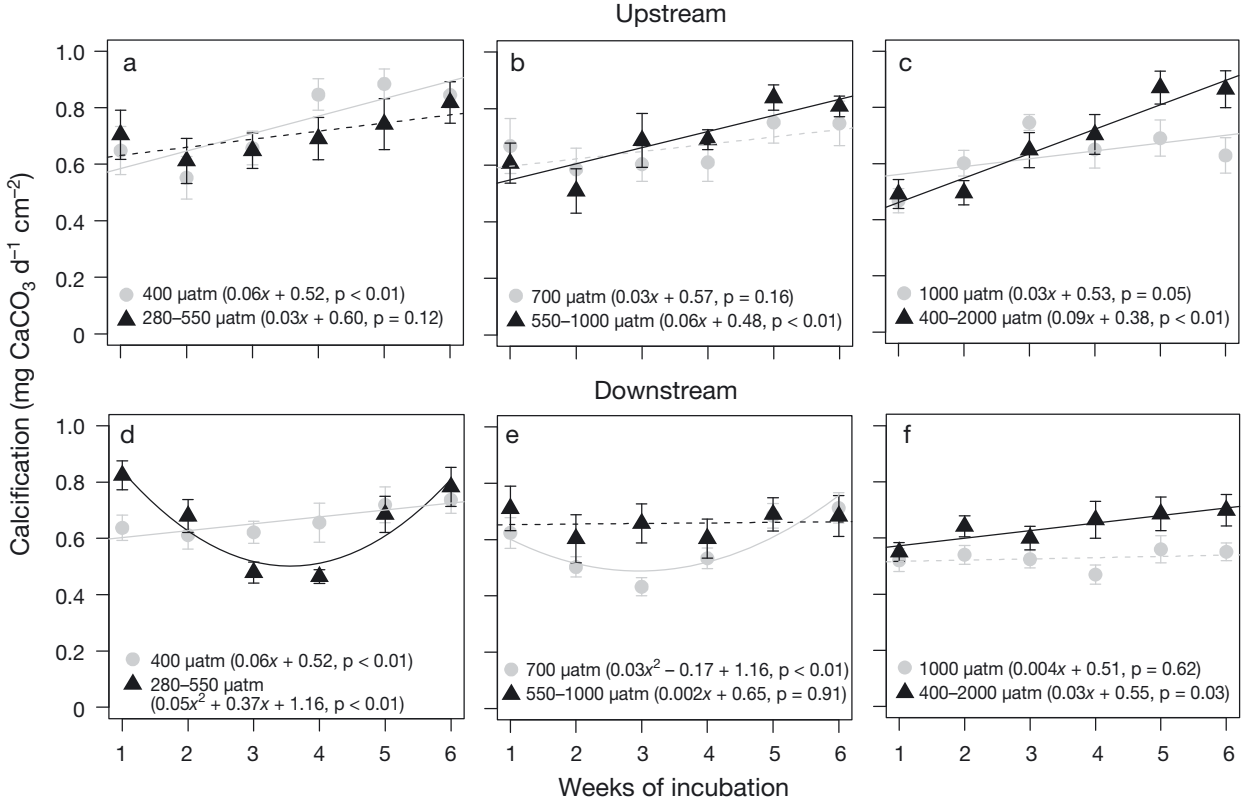

Fig. 3. *Acropora hyacinthus.* Weekly calcification during the 6 wk incubation at constant and oscillating pCO_2. Measurements were made on corals from (a–c) the upstream habitat and (d–f) the downstream habitat. To test the response of calcification to constant and oscillating pCO_2, 6 treatments were used to establish 3 contrasts: 400-constant vs. 280–550-oscillating, 700-constant vs. 550–1000-oscillating, and 1000-constant vs. 400–2000-oscillating. Each point represents the mean weekly calcification rate ± SE (n = 7). The dashed lines represent the linear regressions for which the p-values for the slopes are >0.05. The equations of the linear or non-linear regressions as well as the p-values for the slopes are given in parentheses for each treatment

Table 2. *Acropora hyacinthus*. Comparison of area-normalized calcification, analyzed with a repeated-measures ANOVA with pCO_2 regime as a between-subject effect and Time as a within-subject effect. Significant p-values (<0.05) in **bold**

Site	Treatment	Source	Effect	df	MS	*F*	p
Upstream	400-constant vs.	Between	pCO_2 regime	1	0.027	0.188	0.672
	280–550-oscillating	Within	Time	5	0.137	9.874	**<0.001**
			Time × pCO_2 regime	5	0.154	2.220	0.063
			Residuals	96	0.832		
	700-constant vs.	Between	pCO_2 regime	1	0.016	0.133	0.721
	550–1000-oscillating	Within	Time	5	0.145	8.421	**<0.001**
			Time × pCO_2 regime	5	0.037	2.164	0.070
			Residuals	96	0.017		
	1000-constant vs.	Between	pCO_2 regime	1	0.043	0.702	0.419
	400–2000-oscillating	Within	Time	5	0.186	7.094	**<0.001**
			Time × pCO_2 regime	5	0.065	2.484	**0.041**
			Residuals	96	0.026		
Downstream	400-constant vs.	Between	pCO_2 regime	1	0.003	0.041	0.843
	280–550-oscillating	Within	Time	5	0.109	10.533	**<0.001**
			Time × pCO_2 regime	5	0.069	6.704	**<0.001**
			Residuals	96	0.010		
	700-constant vs.	Between	pCO_2 regime	1	0.123	1.066	0.322
	550–1000-oscillating	Within	Time	5	0.072	9.876	**<0.001**
			Time × pCO_2 regime	5	0.027	3.760	**0.005**
			Residuals	96	0.007		
	1000-constant vs.	Between	pCO_2 regime	1	0.265	5.786	**0.033**
	400–2000-oscillating	Within	Time	5	0.018	2.452	**0.044**
			Time × pCO_2 regime	5	0.012	1.593	0.176
			Residuals	96	0.007		

pCO_2 regime interaction ($p < 0.001$ in both cases, Table 2). Similarly in the contrast between 700-constant and 550–1000-oscillating (Fig. 3e), calcification was not significantly affected by pCO_2 regime ($p = 0.322$, Table 2) but was significantly affected by both Time and the Time × pCO_2 regime interaction ($p < 0.001$ and $p = 0.005$, respectively; Table 2). For the comparison between 1000-constant versus the 400–2000-oscillating treatments (Fig. 3f), calcification was significantly affected by pCO_2 regime ($p = 0.033$, Table 2) due to higher rates of calcification in the 400–2000-oscillating treatment. Time also had a significant effect ($p < 0.044$, Table 2), but not the Time × pCO_2 regime interaction ($p = 0.176$).

Differences in calcification during the last week of incubation

The ANOVA for calcification during the last week of incubation as the dependent variable showed a significant effect of habitat ($p = 0.011$, Table 3, Fig. 4), with calcification 12% lower in downstream versus upstream corals (pooled among treatments). Calcification also was negatively affected by pCO_2 ($p = 0.042$, Table 3, Fig. 4) and the pCO_2 regime (i.e. constant versus oscillating, $p = 0.042$, Table 3, Fig. 4). Calcification was 9% lower in the constant treatments compared to the oscillating treatments (when pooled among habitats and pCO_2 levels). This effect was most striking in the contrast of 1000-constant versus 400–2000-oscillating treatments, for which calcification was 27% and 21% greater in the oscillating versus constant treatment in upstream and downstream corals, respectively.

Table 3. *Acropora hyacinthus*. Comparison of area-normalized calcification (mg $CaCO_3$ d^{-1} cm^{-2}) among treatments at the end of the 6 wk incubation. Area-normalized calcification (dependent variable) was analyzed with a 3-way mixed-model ANOVA in which Habitat and pCO_2 were fixed between plot effects and pCO_2 regime (constant vs. oscillating) was a nested effect. Significant p-values (<0.05) in **bold**

Effect	df	MS	*F*	p
Habitat	1	0.176	6.832	**0.011**
pCO_2	2	0.085	3.308	**0.042**
pCO_2 regime	1	0.110	4.281	**0.042**
Habitat × pCO_2	2	0.005	0.200	0.819
Residuals	77	0.026		

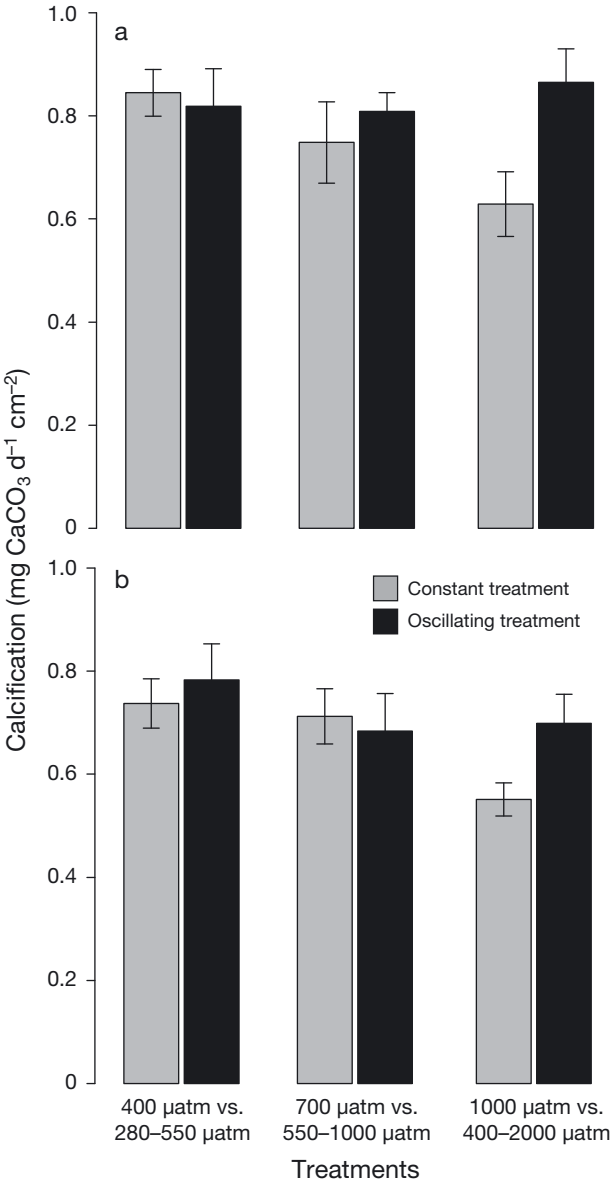

Fig. 4. *Acropora hyacinthus*. Calcification during the last week of the 6 wk incubation at constant and oscillating pCO_2. Calcification rates were determined on corals collected (a) upstream and (b) downstream. Values shown are means ± SE (n = 7)

DISCUSSION

Using a 6 wk incubation with weekly measurements of calcification, we tested for the impact of present and future pCO_2 oscillation on the calcification of *Acropora hyacinthus* and its capacity to acclimate to oscillatory pCO_2. At low pCO_2 (i.e. ≤400 µatm), we did not find differences in calcification under constant and oscillatory pCO_2 at the end of the incubation. In contrast, Dufault et al. (2012) described an increase in calcification for recruits of *Seriatopora caliendrum* maintained for 3 d under oscillatory (420–596 µatm) versus ambient (440 µatm) pCO_2. The difference between the results reported by Dufault et al. (2012) and the present study likely is related to the use of new recruits (i.e. corals ≤6 d old) in the former study that display physiological characteristics strongly shaped by intense selective pressure for rapid growth (Jackson 1977). In addition to using different life stages, lower daytime pCO_2 conditions in our ambient treatment (280 µatm in our study versus 420 µatm in Dufault et al. 2012) may also underlie the different results in our study versus those of Dufault et al. (2012). DIC limitation during the day, associated with the low daytime pCO_2 conditions, could have caused the lack of response of calcification in *A. hyacinthus* to pCO_2 in the ambient treatment (280–550-oscillating). Limitation in the supply of DIC to reef corals has been demonstrated by experiments in which bicarbonate addition promoted daytime calcification and photosynthesis (Furla et al. 2000, Herfort et al. 2008, Jury et al. 2010).

While we did not detect a positive effect of pCO_2 oscillation at intermediate pCO_2 (550–1000 oscillating), corals from both upstream and downstream habitats exhibited higher calcification when maintained in the 400–2000-oscillating treatment than in the 1000-constant treatment. Dufault et al. (2012) proposed that higher calcification rates in juvenile corals exposed to oscillating pCO_2 can be explained by a nighttime storage of DIC in coral tissue. They suggested that in the first hours of light, corals use this stored DIC to enhance calcification, but to date there has been no explicit test of this hypothesis. Our results are consistent with the hypothesized nighttime accumulation of DIC in coral tissues, as calcification was higher in oscillating treatments than in constant treatments following acclimation, particularly in the 400–2000-oscillating treatment.

In addition to the potential for storage of DIC at night by corals exposed to nocturnally elevated pCO_2 (sensu Dufault et al. 2012), there also might be a beneficial consequence of elevated pH during the day when DIC is not limiting. Calcification in corals on average is 3-fold higher in the light compared to the dark (Gattuso et al. 1999) and is linked functionally to photosynthesis (Chalker 1981, Moya et al. 2006, Allemand et al. 2011). Photosynthesis positively affects calcification by increasing pH within coral tissue (Allemand et al. 2011) and can provide additional sources of organic carbon that can be catabolized to provide metabolic energy (Muscatine 1990). In turn, this metabolic energy could be used to support the

active transport of inorganic carbon to the calcification site, and the active removal of protons from the calcification site, both of which would be energetically more costly in a less basic ocean (Allemand et al. 2011, Ries 2011). Export of protons from the corals to the external medium has been hypothesized to be one of the major parameters controlling calcification (Jokiel et al. 2011a,b, Ries 2011). Jokiel (2011b) notably suggested that the export of protons is driven by the limitation of protons at the boundary layer, which in turn is driven by the morphology of corals, boundary layer dynamics, and the seawater pH. Our results lend support to a hypothesized mechanism in which the daytime increase in pH of seawater subject to diel oscillatory pCO_2 favors removal of excess protons resulting from calcification from coral tissue (Jokiel et al. 2011a,b, Ries 2011). Such an effect might be enhanced in the early hours of the morning by DIC stored in the tissue overnight (Dufault et al. 2012), which could be used for both photosynthesis and calcification. Increased photosynthesis could provide the carbon substrates necessary to generate additional metabolic energy through respiration that could be allocated to meeting the costs of calcification. According to Jokiel (2011b), elevated rates of photosynthesis might also cause a decrease in proton concentrations in the seawater surrounding corals that would favor an increased export of protons and thus increased calcification rates. At the scale of whole reefs, the coupling between higher pH during the day, due to high photosynthesis, and calcification has already been described (Shamberger et al. 2011). In addition, one study has demonstrated the favorable role of photosynthesis of algal beds placed upstream from corals in promoting calcification during the day by increasing pH and saturation states (Anthony et al. 2011).

Higher calcification by corals during the day when pCO_2 is lower and pH higher may be sufficient to compensate for low calcification and perhaps skeletal dissolution at night, thereby maintaining positive net calcification in the oscillating pCO_2 treatment when daytime pH is higher. For example, in the 400–2000-oscillating treatment, the low nighttime pH (7.47) likely would depress dark calcification to near 0 in some species (Schneider & Erez 2006, S. Comeau pers. obs.). During the day, high pH (8.07 in the 400–2000-oscillating treatment), which is lower than the high pH in the 280–550-oscillating treatment (pH_T = 8.20), would promote high rates of light-dependent calcification that could be enhanced by the hypothesized supply of DIC generated through nighttime storage. In contrast, in the 1000-constant treatment, light calcification presumably is reduced compared to the 400–2000-oscillating treatment, and dark calcification is slightly higher than in the 400–2000-oscillating treatment. As a result, mean calcification is higher in the 400–2000-oscillating treatment compared to the 1000-constant treatment, as shown in the present study for the corals sampled from upstream and downstream habitats.

Understanding the mechanistic basis of the effects of oscillatory pCO_2 on coral calcification probably will require close attention to the separate effects of pCO_2 on light and dark calcification, which might reflect the effects of different calcification mechanisms in corals. One way light and dark effects could be discriminated would be to perform short-term measurements of light and dark calcification using the alkalinity anomaly technique (Chisholm & Gattuso 1991), which has the resolution to detect even subtle differences in the rates of calcification between light and dark conditions under oscillating and constant pCO_2 conditions. The alkalinity anomaly technique has been used to investigate the response of a coral community from Hawaii incubated under oscillating conditions at ambient or elevated pCO_2 (Andersson et al. 2009). Andersson et al. (2009) measured some dissolution over night in a control treatment where pCO_2 was up to ~700 µatm and a strong dissolution signal in the elevated pCO_2 treatment where pCO_2 was up to ~1500 µatm. In contrast, community calcification was positive during the day in both treatments, and higher in the control where pCO_2 was ~320 µatm (Andersson et al. 2009). These results support our hypothesis that higher calcification by corals during the day when pCO_2 is lower and pH higher may be sufficient to compensate for skeletal dissolution at night explaining the high calcification rates measured in the 400–2000-oscillating treatment.

The abrupt changes in pCO_2 to which the corals were exposed twice a day (07:00 and 19:00 h) during each transfer did not appear to impact the calcification of corals. On the reef, daily oscillations in pCO_2 occur over several hours and organisms are exposed to daily maximum or minimum pCO_2 lasting only a few hours each day. However, due to logistical constraints, we were unable to mimic these gradual changes of pCO_2 in the mesocosms, and instead we utilized abrupt changes that could have resulted in undetected stresses affecting physiological performances (e.g. calcification). However, since calcification was mostly higher in corals incubated in the oscillating treatments, it is reasonable to infer that stresses from abrupt transfer between pCO_2 treatments were small.

In the present study, and in contrast to our recent work (Comeau et al. 2013a), we chose not to acclimate corals to laboratory conditions prior to beginning treatments. This approach was adopted to retain legacy effects on their physiology attributed to the collection habitats of the corals. A trade-off in this experimental design was that corals were acclimating to the laboratory conditions at the same time that they were responding to the treatments. Therefore, treatment effects potentially were influenced by acclimation, although these effects would be similar in all treatments. Acclimation to laboratory conditions was evident in upstream corals in all treatments, and in particular for corals incubated in the treatment with the largest daily variation in pCO_2 and the highest pCO_2 value (400–2000-oscillating). In this treatment, calcification in the oscillating treatment surpassed that of the 1000-constant treatment after 3 wk. In downstream corals, acclimation to the laboratory conditions was less pronounced. Inverse parabolic relationships described the variation in calcification with time in the 280–550-oscillating and 700-constant treatments, and these complex relationships suggest that the responses to laboratory conditions and pCO_2 regimes can be dynamic. Despite the initial reduction in calcification of corals in the 280–550-oscillating treatment, after 3 wk, corals then acclimated quickly as calcification rates returned to their initial level within 6 wk. An acclimation response requiring 3 wk is similar to both the acclimation period of ~2 wk determined in the soft coral *Cladiella* sp. to fully recover from the injuries resulting from fragmentation (Tentori & Allemand 2006) and the photoacclimation period of ~10 d determined in the coral *Turbinaria mesenterina* (Anthony & Hoegh-Guldberg 2003). Acclimation also has been studied in *Pocillipora damicornis* collected from a high flow environment that were allowed to acclimatize for 13 d at different flow regimes (Lesser et al. 1994). During the acclimation period, *P. damicornis* exhibited significant changes in the activities of antioxidant enzymes, photosynthesis, respiration, and carbonate anhydrase activity. These results demonstrate that physiological and biochemical plasticity can allow corals to acclimate quickly to new conditions (i.e. flow regime in Lesser et al. 1994). In the present study, the parabolic shape of the relationship of calcification time as well as the increase in calcification over time in most of the treatments likely resulted from similar acclimation mechanisms to the new pCO_2 regimes.

In corals from the upstream and the downstream habitats, higher calcification rates under oscillating conditions (400 µatm during the day to 2000 µatm at night) versus constant treatments (1000 µatm) were observed once the corals were acclimated both to laboratory conditions and to the extreme oscillations in pCO_2 (i.e. after 4 to 6 wk). Thus far, acclimation of tropical corals to ocean acidification during perturbation experiments has not been demonstrated, yet such an effect clearly would have important implications for interpreting the results of experiments in which corals are exposed to high pCO_2. During one mesocosm experiment, Jokiel et al. (2008) performed 6 consecutive measurements of growth of *Montipora capitata* over 215 d and did not detect acclimation to low aragonite saturation. Similarly, Langdon et al. (2000) did not find any differences in calcification of an artificial coral reef community between short-term and long-term incubations at different aragonite saturation states. To date, acclimation to ocean acidification conditions has been demonstrated only in the aposymbiotic cold water coral *Lophelia pertusa*, in which calcification decreased at high pCO_2 (up to 982 µatm, 7.5°C) over 8 d, but was unaffected by high pCO_2 over 6 mo (Form & Riebesell 2012). These authors proposed that the acclimation of *L. pertusa* to pCO_2 likely was due to a time lag of days to weeks that they inferred was necessary to activate the metabolic pathways required to calcify under high pCO_2. Such metabolic pathways might include the activation of metabolic mechanisms favoring more effective export of protons from the calcification site to the external medium (Ries 2011, McCulloch et al. 2012).

We measured different rates of calcification in corals collected from upstream versus downstream habitats, with calcification 12% higher in corals from the upstream habitat. While among-habitat differences in physiological performance also could arise from fine-grained genetic adaptation, such an effect is unlikely in mass spawning corals (Ayre & Hughes 2000) exposed to strong flow regimes. Unfortunately, it was beyond the scope of this study to address these effects, but regardless of their roles, the response to pCO_2 was similar for corals from the 2 habitats (i.e. there was no interaction between pCO_2 and habitat). This outcome was likely due to the small diel variation in pCO_2 that we detected (69 versus 85 µatm at upstream and downstream habitats), which is much lower than the very large daily variation in pCO_2 recently recorded in reef waters at Lady Elliot Island, Australia (Shaw et al. 2012). While it is currently unknown why upstream–downstream variation in pCO_2 in reef waters in Moorea was so small at the time of measurement, it is possible that contempo-

rary values reflect the low coral cover in the back reef of Moorea (Edmunds et al. 2010, Trapon et al. 2011), the strong cross reef transport of seawater in Moorea that lessens residence time of water over the reef (e.g. Rosman & Hench 2011), and the greater depth of water (~2 m) compared to Lady Elliot Island (~0.4 m) where diel pCO_2 variation is strongly developed (Shaw et al. 2012).

Our study, which is the first to investigate the effects of present and potential future diel pCO_2 oscillations on reef corals, demonstrates both acclimation to the experimental conditions over a 6 wk period, and null or positive effects of pCO_2 oscillations on calcification rates. After 6 wk and for both upstream and downstream corals, there was no effect of pCO_2 on the calcification of corals in the oscillating treatments. The increased resistance of *Acropora hyacinthus* exposed to large pCO_2 oscillation (particularly in the 400–2000-oscillating treatment) compared to the response of corals under constant pCO_2 conditions suggests that reef corals may be more resistant to future OA conditions in locations where diel variation in seawater pCO_2 is pronounced. In addition to the disparities in the responses of corals to OA as a result of species-specific effects (Pandolfi et al. 2011, Comeau et al. 2013a) as well as environmental factors such as food availability (e.g. Holcomb et al. 2010, Edmunds 2011, Comeau et al. 2013b) and light (Dufault et al. 2013), our study shows that the response of calcifiers likely will also be habitat-dependent and affected by the extent to which pCO_2 levels oscillate on a daily cycle.

Acknowledgements. We thank the NSF for financial support (OCE 10-41270) and the Moorea Coral Reef Long Term Ecological Research site (OCE 04-17413 and 10-26852) for logistic support. We thank 3 anonymous reviewers for comments that improved an earlier draft of this paper. This is contribution 208 of the California State University, Northridge, Marine Biology Program.

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Editorial responsibility: Charles Birkeland, Honolulu, Hawaii, USA

Submitted: September 10, 2013; Accepted: December 10, 2013
Proofs received from author(s): March 1, 2014